ARCHITECT LH

REF 2P40-25
REF 2P40-35

en
LH
2P40
G6-0462 / R05
B2P400

Read Highlighted Changes: Revised October 2015.

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

NAME

ARCHITECT LH

INTENDED USE

The ARCHITECT LH assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of human luteinizing hormone (LH) in human serum and plasma.

SUMMARY AND EXPLANATION OF THE TEST

Human luteinizing hormone (LH, lutropin) is a glycoprotein hormone with two dissimilar subunits (α and β). The α-subunit is essentially identical to the α-subunits of follicle stimulating hormone (FSH, follitropin), thyroid stimulating hormone (TSH, thyrotropin), and human chorionic gonadotropin (hCG).[1-4] The β-subunit is considerably different from that of FSH and TSH.[1, 4, 5] However, the β-subunits of LH and hCG are very similar.[1, 5, 6]

LH, together with FSH, is secreted by the gonadotroph cells in the pituitary[5, 7] in response to the secretion of the gonadotropin releasing hormone (LHRH, GnRH) from the medial basal hypothalamus.[8-10] Ovarian steroids, principally estrogens, modulate the secretion of LH and FSH which in turn regulate the menstrual cycle in females. When the follicle and the ovum contained within it, reach maturity, a surge of LH causes the follicle to rupture releasing the ovum. The follicular remnant is transformed into a corpus luteum, which secretes progesterone and estradiol. During the follicular and luteal phases, LH concentrations are much lower than the levels observed at the time of the LH surge. During the follicular and luteal phases, the estrogens exert a negative feedback on the release of LH. Shortly before the mid-cycle surge in LH, ovarian steroids, specifically estradiol, exert a positive feedback on the release of LH.[11-13]

Determination of the concentration of LH is essential for the prediction of ovulation, in the evaluation of infertility, and the diagnosis of pituitary and gonadal disorders.[11, 14] Increasing concentrations of LH precede ovulation and in cases in which the period of optimal fertility needs to be defined for the timing of intercourse or artificial insemination, daily concentrations of LH are important for the prediction of ovulation. More frequent sampling is required if the precise time of follicular rupture is needed for egg aspiration for *in vitro* fertilization.[15]

At menopause, or following ovariectomy in women, concentrations of estrogens decline to low levels. The lowered concentrations of estrogens result in a loss of the negative feedback on gonadotropin release. The consequence is an increase in the concentrations of LH and FSH.[11, 15, 16]

The primary role of LH in the male is to stimulate the production of testosterone by the Leydig cells. LH, through the production of testosterone together with FSH, regulates spermatogenesis in the Sertoli cells of the seminiferous tubules of the testes. Testosterone exerts a negative feedback on the release of LH.[14]

In sexually mature adults, gonadotropin deficiency is usually an early indication of the development of panhypopituitarism. Low concentrations of LH, FSH, and steroids are observed with this disorder. In contrast, gonadotropin secreting tumors of the hypothalamus and pituitary result in elevated concentrations of LH and FSH.[15]

Gonadal failure, a cause of infertility, is indicated by elevated concentrations of LH and FSH accompanied by low concentrations of gonadal steroids.[11, 14, 15] In the female, elevated concentrations of LH can indicate primary amenorrhea,[11] menopause,[11, 15, 16] premature ovarian failure,[15, 17] polycystic ovarian syndrome,[17, 18] hypergonadotropic hypogonadism,[11, 15] or ovulation. In the male, elevated concentrations of LH can result from primary testicular failure, seminiferous tubule dysgenesis (Klinefelter's syndrome), Sertoli cell failure, anorchia, or hypergonadotropic hypogonadism.[19, 20]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT LH assay is a two-step immunoassay for the quantitative determination of luteinizing hormone (LH) in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex.

1. Sample and anti-β LH coated paramagnetic microparticles are combined. The LH present in the sample binds to the anti-β LH coated microparticles.
2. After washing, anti-α LH acridinium-labeled conjugate is added to create a reaction mixture.
3. Following another wash cycle, Pre-Trigger and Trigger Solutions are added to the reaction mixture.
4. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of LH in the sample and the RLUs detected by the ARCHITECT iSystem optics.

For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

REAGENTS

Kit Contents

ARCHITECT LH 2P40

NOTE: Some kit sizes are not available in all countries or for use on all ARCHITECT iSystems. Please contact your local distributor.

REF	2P40-25	2P40-35
Σ	100	500
MICROPARTICLES	1 x 6.6 mL	1 x 27.0 mL
CONJUGATE	1 x 5.9 mL	1 x 26.3 mL

MICROPARTICLES Anti-β LH (mouse, monoclonal) antibody coated microparticles in HEPES buffer with protein (bovine, mouse) stabilizers. Minimum concentration: 0.04% solids. Preservative: ProClin 300.

CONJUGATE Anti-α LH (mouse, monoclonal) acridinium-labeled conjugate in MES buffer with protein (bovine, casein) stabilizers. Minimum concentration: 170 ng/mL. Preservatives: ProClin 300 and ProClin 950.

Other Reagents

MULTI-ASSAY MANUAL DILUENT 1 x 100 mL ARCHITECT Multi-Assay Manual Diluent, REF 7D82-50, containing phosphate buffered saline solution. Preservative: antimicrobial agent.

PRE-TRIGGER SOLUTION ARCHITECT Pre-Trigger Solution containing 1.32% (w/v) hydrogen peroxide.

TRIGGER SOLUTION ARCHITECT Trigger Solution containing 0.35 N sodium hydroxide.

WASH BUFFER ARCHITECT Wash Buffer containing phosphate buffered saline solution. Preservatives: antimicrobial agents.

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use

Safety Precautions

CAUTION: This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[21-24]

The following warnings and precautions apply to: MICROPARTICLES / CONJUGATE	
	
WARNING	Contains methylisothiazolones.
H317	May cause an allergic skin reaction.
Prevention	
P261	Avoid breathing mist / vapors / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.

For a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

Reagent Handling

- Do not use reagent kits beyond the expiration date.
- **Do not pool reagents within a kit or between kits.**
- Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. For microparticle mixing instructions, refer to the **PROCEDURE, Assay Procedure** section of this package insert.
- **Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.**
  - To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.
  - Once a septum has been placed on an open reagent bottle, **do not invert the bottle** as this will result in reagent leakage and may compromise assay results.
  - Over time, residual liquids may dry on the septum surface. These are typically dried salts and have no effect on assay efficacy.

For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Reagent Storage

When stored and handled as directed, reagents are stable until the expiration date.

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened/ Opened*	2-8˚C	Until expiration date	May be used immediately after removal from 2-8˚C storage. Store in upright position.
On board	System temperature	30 days	Discard after 30 days. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.

* Reagents may be stored on or off the ARCHITECT iSystem. If reagents are removed from the system, store them at 2-8˚C (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright. **If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded.** For information on unloading reagents, refer to the ARCHITECT System Operations Manual, Section 5.

Indications of Reagent Deterioration

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

The ARCHITECT LH assay file must be installed on the ARCHITECT iSystem from an ARCHITECT iSystem Assay CD-ROM prior to performing the assay.

For detailed information on assay file installation and viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.

For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.

For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.

Alternate Result Units

Edit assay parameter "Result concentration units" to select an alternate unit.

Conversion formula:

(Concentration in Default result unit) x (Conversion factor) = (Concentration in Alternate result unit)

Default result unit	Conversion factor	Alternate result unit
mIU/mL	1	IU/L

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

Verified specimen types to be used with this assay:

Specimen Types	Collection Tubes
Human serum	Serum
	Serum separator tubes (SST)
Human plasma	Potassium-EDTA
	Sodium-Heparin

- Other specimen collection tube types have not been tested with this assay.
- Performance has not been established for the use of cadaveric specimens or the use of body fluids other than human serum or plasma.
- Liquid anticoagulants may have a dilution effect resulting in lower concentrations for individual patient specimens.
- The instrument does not provide the capability to verify specimen type. It is the responsibility of the operator to verify that the correct specimen types are used in the assay.

Specimen Conditions

- Do not use specimens with the following conditions:
  - heat-inactivated
  - pooled
  - grossly hemolyzed (> 500 mg/dL hemoglobin)
  - obvious microbial contamination
- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for collection tubes. Gravity separation is not sufficient for specimen preparation.
- Mix thawed specimens thoroughly by low speed vortexing or by inverting 10 times. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous.
- To ensure consistency in results, specimens must be transferred to a centrifuge tube and centrifuged at ≥ 10,000 RCF (Relative Centrifugal Force) for 10 minutes before testing if
  - they contain fibrin, red blood cells, or other particulate matter,
  - they require repeat testing, or
  - they were frozen and thawed.
- Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.
- Inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

Specimen Storage

Specimen Type	Storage Temperature	Maximum Storage Time
Serum/Plasma	2-8°C	≤ 7 days

If testing will be delayed more than 24 hours, remove serum or plasma from the clot, red blood cells, or separator gel.

If testing will be delayed more than 7 days, store frozen (< - 10°C).

Specimens that encountered three freeze/thaw cycles showed no performance difference.

Avoid multiple freeze/thaw cycles.

Specimen Shipping

- Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.
- Do not exceed the storage limitations listed above.

PROCEDURE

Materials Provided

2P40 ARCHITECT LH Reagent Kit

Materials Required but not Provided

- ARCHITECT LH Assay file obtained from the ARCHITECT iSystem e-Assay CD-ROM found on www.abbottdiagnostics.com.
- 2P40-01 ARCHITECT LH Calibrators
- Abbott Immunoassay-MCC (Liquid) or other control material
- 7D82-50 ARCHITECT Multi-Assay Manual Diluent
- ARCHITECT Pre-Trigger Solution
- ARCHITECT Trigger Solution
- ARCHITECT Wash Buffer
- ARCHITECT Reaction Vessels
- ARCHITECT Sample Cups
- ARCHITECT Septum
- ARCHITECT Replacement Caps
- Pipettes or pipette tips (optional) to deliver the volumes specified on the patient or control order screen.

For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.

Assay Procedure

- Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. After the first time the microparticles have been loaded, no further mixing is required.
  - **Invert the microparticle bottle 30 times.**
  - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
  - **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.**
  - Once the microparticles have been resuspended, place a septum on the bottle. For instructions about placing septums on bottles, refer to the **Reagent Handling** section of this package insert.
- Load the reagent kit on the ARCHITECT iSystem.
  - Verify that all necessary reagents are present.
  - Ensure that septums are present on all reagent bottles.
- Order calibration, if necessary.
  - For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.
- Order tests.
  - For information on ordering patient specimens and controls and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
- Minimum sample cup volume is calculated by the system and printed on the Orderlist report. To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.

  Maximum number of replicates sampled from the same sample cup: 10
  - Priority:

    Sample volume for first test: 75 µL

    Sample volume for each additional test from same sample cup: 25 µL

- ≤ 3 hours on board:

  Sample volume for first test: 150 µL

  Sample volume for each additional test from same sample cup: 25 µL
- > 3 hours on board: Additional sample volume is required. For information on sample evaporation and volumes, refer to the ARCHITECT System Operations Manual, Section 5.
- If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.

- Prepare ARCHITECT LH Calibrators.
  - Mix calibrator(s) by gentle inversion before use.
  - Hold bottles **vertically** and dispense recommended volumes into each respective sample cup.
  - Recommended volumes:

    for each calibrator: 4 drops
  - Follow the manufacturer's instructions for preparation of commercially available control material.
- Load samples.
  - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN.
- For additional information on principles of operation, refer to the ARCHITECT System Operations Manual, Section 3.
- For optimal performance, it is important to perform routine maintenance as described in the ARCHITECT System Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

Specimen Dilution Procedures

Specimens with a LH value exceeding 250.00 mIU/mL (>250.00 IU/L) are flagged with the code ">250.00" and may be diluted using either the Automated Dilution Protocol or the Manual Dilution Procedure.

Only specimens with a concentration of greater than 2.00 mIU/mL (2.00 IU/L) should be diluted.

Automated Dilution Protocol

The system performs a 1:4 dilution of the specimen and automatically calculates the concentration of the specimen before dilution and reports the result.

Specimens exceeding 1000.00 mIU/mL (1000.00 IU/L) are flagged with the code ">1000.00" when run using the Automated Dilution Protocol.

Manual Dilution Procedure

Suggested dilution: 1:4

It is recommended that dilutions not exceed 1:4.

1. Add 40 µL of the patient specimen to 120 µL of ARCHITECT Multi-Assay Manual Diluent.
2. The operator must enter the dilution factor in the Patient or Control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution and report the result.

For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

Calibration

- Test Calibrators A-F in duplicate. The calibrators should be priority loaded.

  To evaluate the calibration of this assay using commercially available controls, a single sample of all levels of controls should be tested to evaluate the assay calibration. Ensure that assay control values are within the established ranges.
- Calibration Range: 0.00 - 250.00 mIU/mL (0.00 – 250.00 IU/L).
- Once an ARCHITECT LH calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - A reagent kit with a new lot number is used or
  - Controls are out of range.
- For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

Quality Control Procedures

- The recommended control requirement for the ARCHITECT LH assay is that a single sample of each control level be tested once every 24 hours each day of use. If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow your laboratory-specific procedures or your federal, state and/or local accrediting agency requirements or regulations.
- Additional controls may be tested in accordance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control policy.
- Each laboratory should establish control ranges to monitor the acceptable performance of the assay. If a control is out of its specified range, the associated sample results are invalid and the samples must be retested. Recalibration may be indicated.
- When using commercially available controls, each laboratory should establish its own concentration ranges for new control lots at each clinically relevant control level. This can be accomplished by assaying a minimum of 20 replicates over several (3-5) days. Sources of variation that can be expected should be included in this study in order to be representative of future system performance. These may include:
  - Multiple stored calibrations
  - Multiple reagent lots
  - Multiple calibrator lots
  - Multiple processing modules
  - Data points collected at different times of the day
- Commercial controls should be used according to the guidelines and recommendations of the control manufacturer. In addition, each laboratory should establish its own concentration ranges for new control lots at each control level employed. These ranges should be established according to your laboratory's quality control policy and/or any local, state, and/or federal regulations or accreditation requirements. Concentration ranges provided in the control package insert should be used only for guidance.
- For any control material in use, the laboratory should ensure that the matrix of the control material is suitable for use in the assay per the assay package insert.
- Refer to Clinical and Laboratory Standards Institute (CLSI) Document C24-A3 or other published guidelines for general quality control recommendations.[25]

Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B.

The ARCHITECT LH assay belongs to method group 6.

RESULTS

Calculation

The ARCHITECT LH assay utilizes a 4 Parameter Logistic Curve fit data reduction method (4PLC, Y-weighted) to generate a calibration curve.

The ARCHITECT iSystem calculates the Calibrator A through F mean chemiluminescent signal from two Calibrator A through F replicates, generates a calibration curve and stores the result.

For information on alternate result units, refer to the **INSTRUMENT PROCEDURE, Alternate Result Units** section of this package insert.

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the ARCHITECT System Operations Manual, Section 5.

Measuring Interval (Reportable Range)

Measuring interval is defined as the range of values in mIU/mL which meets the limits of acceptable performance for both imprecision and bias for an undiluted sample. For the verification studies described in this package insert, the range was 0.09 mIU/mL (Limit of Quantitation - LoQ) to 250.00 mIU/mL. When using the manual or automated dilution procedure, the assay can report values up to 1000.00 mIU/mL.

LIMITATIONS OF THE PROCEDURE

- Results should be used in conjunction with other data; e.g., symptoms, results of other tests, and clinical impressions.
- If the LH results are inconsistent with clinical evidence, additional testing is recommended to confirm the result.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits such as ARCHITECT LH that employ mouse monoclonal antibodies. Additional information may be required for diagnosis.[26, 27]
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference, and anomalous values may be observed. Additional information may be required for diagnosis.[28]

EXPECTED VALUES

The suggested normal range for the ARCHITECT LH assay represents the LH values obtained from 199 normal males, 124 postmenopausal females (not on hormone replacement therapy - HRT), and 64 normally menstruating females. For this study, the follicular phase was defined as the period of time from 10 to 4 days prior to the mid-cycle peak. The luteal phase was defined as the period of time from 4 to 10 days following the mid-cycle peak. Cycle days were synchronized to the mid-cycle peak, the day on which the LH concentration was most elevated. The results are presented in the following table.

		LH Values (mIU/mL)		
		Median/	Central 95% of Data	
	n	Mean*	Lower Limit	Upper Limit
Normal Males	199	2.96	0.57	12.07
Normally Menstruating Females				
Follicular Phase	303	3.98	1.80	11.78
Mid-Cycle Peak	64	26.00*	7.59	89.08
Luteal Phase	294	2.79*	0.56	14.00
Postmenopausal Females				
Without HRT	124	25.73*	5.16	61.99

It is recommended that each laboratory establish its own reference range that is appropriate for the laboratory's patient population (i.e., a normal range that reflects the type of specimen and demographic variables such as age and sex, as applicable).

SPECIFIC PERFORMANCE CHARACTERISTICS

Data in the section **SPECIFIC PERFORMANCE CHARACTERISTICS** were generated using the ARCHITECT i2000SR System.

Assay results obtained in individual laboratories may vary from data presented.

Precision

The ARCHITECT LH assay is designed to have an assay imprecision of ≤ 7% total (within laboratory) CV for LH values ≤ 70 mIU/mL and ≤ 10% total CV for LH values > 70 mIU/mL. For values < 1 mIU/mL down to 0.5 mIU/mL the assay is designed to have an assay imprecision of ≤ 0.07 SD.

A study was performed based on guidance from the CLSI protocol EP5-A2[29]. Four serum based panels (panel member 1-4) and four plasma based panels (panel member 5-8) were assayed using two lots of reagents, on two instruments, in replicates of three at two separate times per day for 20 days. Data from this study are summarized in the following table.**

Panel				Mean Conc.	Within Run		Within Laboratory (Total)	
Member	Reagent Lot	Instrument	n	(mIU/mL)	SD	%CV	SD	%CV
1	1	1	120	3.52	0.099	2.8	0.101	2.9
	1	2	120	3.79	0.122	3.2	0.130	3.4
	2	1	120	3.39	0.068	2.0	0.087	2.6
	2	2	120	3.40	0.124	3.6	0.131	3.9
2	1	1	119	16.01	0.297	1.9	0.389	2.4
	1	2	120	17.18	0.487	2.8	0.533	3.1
	2	1	120	15.84	0.307	1.9	0.464	2.9
	2	2	120	15.56	0.454	2.9	0.502	3.2
3	1	1	119	47.69	1.086	2.3	1.291	2.7
	1	2	120	51.03	1.522	3.0	1.989	3.9
	2	1	120	47.82	1.012	2.1	1.442	3.0
	2	2	120	46.55	1.200	2.6	1.633	3.5
4	1	1	120	222.58	4.913	2.2	7.937	3.6
	1	2	120	239.29	7.147	3.0	7.927	3.3
	2	1	120	228.31	5.188	2.3	8.962	3.9
	2	2	119	220.87	4.888	2.2	6.160	2.8
5	1	1	120	1.00	0.029	2.9	0.035	3.5
	1	2	120	1.08	0.037	3.5	0.044	4.1
	2	1	119	0.95	0.023	2.4	0.028	2.9
	2	2	120	0.94	0.028	3.0	0.031	3.3
6	1	1	120	5.21	0.109	2.1	0.230	4.4
	1	2	119*	5.58	0.171	3.1	0.180	3.2
	2	1	120	5.01	0.121	2.4	0.149	3.0
	2	2	120	5.00	0.134	2.7	0.187	3.7
7	1	1	120	46.23	0.787	1.7	1.073	2.3
	1	2	120	49.17	1.581	3.2	1.827	3.7
	2	1	120	46.08	1.271	2.8	1.738	3.8
	2	2	120	45.76	1.032	2.3	1.227	2.7
8	1	1	120	93.33	2.949	3.2	8.152	8.7
	1	2	120	96.54	3.524	3.6	5.342	5.5
	2	1	120	93.21	2.942	3.2	8.316	8.9
	2	2	120	91.25	2.295	2.5	4.871	5.3

* One aberrant result was identified for panel member 6 and the within run and total imprecision were calculated with this replicate excluded.

** Representative data; results in individual laboratories may vary from these data.

Accuracy by Recovery

The ARCHITECT LH assay is designed to have a mean recovery of 100% ± 8% when analyzing samples spiked with known concentrations of LH at each level tested across the range of 10 to 70 mIU/mL. A study was performed where known concentrations of LH were added to 15 specimens with different endogenous LH levels. Human pituitary luteinizing hormone (lyophilized, > 95% purity) diluted in normal human male serum was used to spike stock solutions. The concentration of LH was obtained using the ARCHITECT LH assay and the resulting percent recovery was calculated. Data from this study are summarized in the following table.*

Specimen	Endogenous Level (mIU/mL)	LH Added (mIU/mL)	Value Obtained (mIU/mL)	%Recovery[a]
1	1.99	9.93	12.67	107.5
2	2.04	11.91	15.15	110.1
3	3.95	9.86	13.28	94.6
4	5.21	11.77	15.64	88.6
5	8.12	4.86	12.76	95.4
6	8.34	4.85	13.08	97.8
7	10.28	19.85	32.13	110.1
8	11.76	24.78	38.40	107.5
9	12.02	54.50	65.64	98.4
10	19.37	39.43	58.45	99.1
11	20.14	44.32	62.44	95.5
12	28.84	24.46	56.02	111.1
13	24.06	34.35	58.41	100.0
14	31.67	8.90	39.37	86.5
15	43.37	19.32	65.83	116.2
Average Recovery: 101.2%				

* Representative data; results in individual laboratories may vary from these data.

$$^{a}\%\ \text{Recovery} = \frac{\text{Value obtained - Endogenous level}}{\text{LH added}} \times 100$$

Linearity

Based on guidance from CLSI protocol EP6-A[30], a study was performed to establish the linear range of the ARCHITECT LH assay. The assay demonstrated linearity within the range of LoQ to 250.00 mIU/mL with an absolute deviation from linearity of ≤ 1 mIU/mL for samples within LoQ and 10 mIU/mL, ≤ 11% for samples within 10 and 70 mIU/mL, and ≤ 15% for samples above 70 mIU/mL.

Sensitivity

Limit of Blank, Limit of Detection and Limit of Quantitation

The ARCHITECT LH assay is designed to have a Limit of Quantitation (LoQ) of ≤ 0.5 mIU/mL.

The Limit of Quantitation (LoQ) of the ARCHITECT LH assay was determined based on guidance from CLSI protocol EP17-A[31]. The LoQ is defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable error of ≤ 22%. The study was performed with 4 blank (zero-level) samples and 8 samples with LH concentrations ranging from 0.05 to 0.11 mIU/mL. These samples were tested over a period of minimum 3 days using 2 reagent lots and 2 instruments. The observed LoQ for the ARCHITECT LH assay was 0.09 mIU/mL.

The Limit of Blank (LoB) and Limit of Detection (LoD) of the ARCHITECT LH assay were determined, using proportions of false positives (α) less than 5% and false negatives (β) less than 5%. These determinations were performed using 4 blank (240 replicates) and 8 low level LH samples (478 replicates); LoB = 0.01 mIU/mL and LoD = 0.03 mIU/mL.

Specificity

The specificity of the ARCHITECT LH assay was determined by studying potential cross-reacting hormones (FSH at 150 mIU/mL, TSH at 100 µIU/mL, and hCG at 200,000 mIU/mL).

A study was performed with the ARCHITECT LH assay and data are summarized in the following table*. Aliquots of ARCHITECT LH Calibrator A, containing essentially no LH (0 mIU/mL), as well as a pool of normal male serum (≤ 10 mIU/mL) and spiked normal male serum samples (50-70 mIU/mL) were supplemented with potential cross-reactants at the concentrations listed and tested for LH.

Cross- Reactant	Concentration	LH Analyte Level mIU/mL	% Cross- Reactivity[a]
FSH	169 mIU/mL	0	0.01
	179 mIU/mL	≤ 10	0.00
	162 mIU/mL	50-70	0.15
TSH	124 µIU/mL	0	0.00
	126 µIU/mL	≤ 10	0.01
	137 µIU/mL	50-70	-0.69
hCG	209,770 mIU/mL	0	0.01
	218,532 mIU/mL	≤ 10	0.01
	206,844 mIU/mL	50-70	0.01

* Representative data; results in individual laboratories may vary from these data.

$$^{a}\%\ \text{Cross-Reactivity} = \frac{\text{mean LH test concentration} - \text{mean LH reference concentration}}{\text{concentration of cross-reactant}} \times 100$$

Interference

Potential interference in the ARCHITECT LH assay from hemoglobin, bilirubin, triglycerides, and protein is designed to be within ± 8% in the range of 10 to 70 mIU/mL.

Interference was demonstrated by a study based on guidance from the CLSI protocol EP7-A2[34]. Data from this study are summarized in the following table*.

Potentially Interfering Substance	Concentration	% Change in Measured Concentration	
		10-20 mIU/mL	50-70 mIU/mL
Bilirubin	≥20 mg/dL	0	-1
Protein	≥12 g/dL	-8	-8
Triglycerides	≥3000 mg/dL	0	-2
Hemoglobin	≥500 mg/dL	1	0

* Representative data; results in individual laboratories may vary from these data.

For interference by rheumatoid factor (RF) and HAMA the ARCHITECT LH assay is designed to have a recovery of 100 ± 8% when analyzing RF or HAMA positive samples spiked with known amounts of LH across the range of 10 to 70 mIU/mL. Results are summarized in the following table*.

Potentially Interfering Substance	Mean % Recovery		
	10-20 mIU/mL	50-70 mIU/mL	Overall
HAMA	101	97	99
RF	97	90	94

* Representative data; results in individual laboratories may vary from these data.

Method Comparison

Correlation

The ARCHITECT LH (2P40) assay is designed to have a slope of 0.9 to 1.15 and a correlation coefficient of ≥ 0.95 for samples across the range of 0 to 250 mIU/mL when compared to the ARCHITECT LH (6C25) assay.

A study was performed with the ARCHITECT LH assay based on guidance from the CLSI protocol EP9-A2-IR[32], where 107 unique specimens were tested in replicates of two on both the investigational and comparator assays. Regression analysis was performed on the mean of the two replicates using the Passing-Bablok[33] and least squares methods. Data from this study are summarized in the following table*.

Regression Method	n	Slope	Intercept	Correlation Coefficient
Least Squares	107	0.98	1.56	0.99
Passing-Bablok [a]	107	1.04	-0.27	0.99

[a] A linear regression method with no special assumptions regarding the distribution of the samples and measurement errors.

* Representative data; variables such as differences in sampling size and sample population may impact the correlation of the assay, therefore, results in individual laboratories may vary from these data.

In this evaluation, specimen concentrations ranged from 1.00 mIU/mL to 207.80 mIU/mL with the ARCHITECT LH (2P40) assay and from 1.11 mIU/mL to 236.20 mIU/mL with the ARCHITECT LH (6C25) assay. The specimens included in the study were sourced from external commercial vendors and derived from different sample categories (normal males, normally menstruating females, and postmenopausal females without HRT). Since samples greater than 100 mIU/mL are rarely expected in normal populations, 7 normal male samples were spiked with LH containing material to cover the upper measuring range of the assay.

BIBLIOGRAPHY

1. Pierce JG, Parsons TF. Glycoprotein Hormones: Structure and Function. *Annu Rev Biochem* 1981:50:465-495.
2. Shome B, Parlow AF. Human Follicle Stimulating Hormone (hFSH): First Proposal for the Amino Acid Sequence of the α-subunit and First Demonstration of its α-subunit identity with an α-subunit of human Luteinizing Hormone (hLHα) *J Clin Endocrinol Metab* 1974; 39:199-202.
3. Sairam MR, Li CH. Human Pituitary Thyrotropin. Isolation and Chemical Characterization of its Subunits. *Biochem Biophys Res Commun* 1973; 51:336-342.
4. Vaitukaitis JL, Ross GT, Braunstein GD, et al. Gonadotropins And Their Subunits: Basic and Clinical Studies. *Recent Prog Horm Res* 1976; 32:289-331.
5. Bishop WH, Nureddin A, Ryan RJ. Pituitary Luteinizing and Follicle Stimulating Hormones. In: Parons JA, editor. Peptide Hormones. Baltimore: University Park Press, 1976; 273-298.
6. Keutmann HT, Williams RM, Ryan RJ. Structure of Human Luteinizing Hormone Beta Subunit: Evidence for a Related Carboxyl-Terminal Sequence Among Certain Peptide Hormones. *Biochem Biophys Res Commun* 1979; 90:842-848.
7. Daughaday WH. The Adenohypophysis. In: Wilson JD and Foster DW, editors. Williams Textbook of Endorcrinology. Philadelphia: Saunders, 1985; 80-83.
8. Schally AV, Arimura A, Kastin AJ, et al. Gonadotropin Releasing Hormone: One Polypeptide Regulates Secretion of Luteinizing and Follicle Stimulating Hormones. *Science* 1971; 173:1036-1038.
9. Harris GW, Naftolin F. The Hypothalamus and Control of Ovulation. *Br Med Bull* 1970; 26:3-9.
10. Knobil E. The Neuroendocrine Control of the Menstrual Cycle. *Recent Prog Horm Res* 1980; 36:53-88.
11. Ross GT. Disorders of the Ovary and Female Reproductive Tract. In: Wilson JD, Foster DW, editors. *Williams Textbook of Endocrinology*. Philadelphia: WB Saunders Co. 1985:206-258.
12. Vande Wiele RL, Bogumil J, Dyrenfurth I, et al. Mechanisms Regulating the Menstrual Cycle in Women. *Recent Prog Horm Res* 1970; 26:63-103.
13. Bonnar J. Gynaecology and Obstetrics; The Hypothalamus and Reproductive Function. In: Scott RB and Walker RM, editors. *The Medical Annual*. Bristol (England): J Wright & Sons 1973; 251-258.
14. Griffin JE, Wilson JD. Disorders of the Testes and Male Reproductive Tract. In: Wilson JD and Foster DW, editors. Williams Textbook of Endocrinology. Philadelphia: Saunders 1985; 259-311.
15. Beastall GH, et al. Assays for Follicle Stimulating Hormone and Luteinizing Hormone: Guidelines for the Provision of a Clinical Biochemistry Service. *Ann Clin Biochem* 1987;24:246-262.
16. Judd HL. Hormonal Dynamics Associated with the Menopause. *Clin Obstet Gynecol* 1976;19:775-788.
17. Kletzky OA, Davajan V. Differential Diagnosis of Secondary Amenorrhea. In: Mishell DR Jr, Brenner PF, editors. Management of Common Problems in Obstetrics and Gynecology. Oradell: *Medical Economics Books* 1983; 352-356.
18. Lachelin G. The Polycystic Ovary Syndrome. In: Studd J, editor. *Prog Obstet Gynecol*. Edinburgh: Churchill Livingstone 1984; 290-301.
19. Franchimont P. Human Gonadotropin Secretion in Male Subjects. In: James VHT, Serio M and Martini L, editors. The Endocrine Function of the Human Testis. New York: Academic Press, 1973:439-458.
20. Marshall JC. Investigative Procedures. Pituitary-Gonadal Relations in Infancy. *J Clin Endocrinol Metab* 1975; 4:545-551.
21. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Bloodborne pathogens.
22. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; December 2009.
23. World Health Organization. *Laboratory Biosafety Manual*. 3rd ed. Geneva: World Health Organization; 2004.
24. Clinical and Laboratory Standards Institute (CLSI). *Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Fourth Edition.* CLSI Document M29-A4. Wayne, PA: CLSI; 2014.
25. Clinical and Laboratory Standards Institute (CLSI). *Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline—Third Edition.* CLSI Document C24-A3. Wayne, PA: CLSI; 2006.
26. Primus FJ, Kelley EA, Hansen HJ, et al. "Sandwich"-type immunoassay of carcinoembryonic antigen in patients receiving murine monoclonal antibodies for diagnosis and therapy. *Clin Chem* 1988;34(2):261-264.
27. Schroff RW, Foon KA, Beatty SM, et al. Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy. *Cancer Res* 1985;45(2):879-885.
28. Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. *Clin Chem* 1988;34(1):27-33.
29. National Committee for Clinical Laboratory Standards (NCCLS). *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition.* NCCLS Document EP5-A2. Wayne, PA: NCCLS; 2004.
30. National Committee for Clinical Laboratory Standards (NCCLS). *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline.* NCCLS Document EP6-A. Wayne, PA: NCCLS; 2003.
31. National Committee for Clinical Laboratory Standards (NCCLS). *Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline.* NCCLS Document EP17-A. Wayne, PA: NCCLS; 2004.
32. Clinical and Laboratory Standards Institute. *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Second Edition (Interim Revision).* CLSI Document EP9-A2-IR. Wayne, PA: Clinical and Laboratory Standards Institute, 2010.
33. Passing H, Bablok W. A new biometrical procedure for testing the equality of measurements from two different analytical methods. Application of linear regression procedures for method comparison studies in clinical chemistry, Part I. *J Clin Chem Clin Biochem* 1983;21(11):709–720.
34. Clinical and Laboratory Standards Institute (CLSI). *Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition.* CLSI Document EP7-A2. Wayne, PA: CLSI; 2005.

Key to Symbols

	Consult instructions for use
	Manufacturer
	Sufficient for
	Temperature limitation
	Use by/Expiration date
CONJUGATE	Conjugate
CONTROL NO.	Control Number
DISTRIBUTED IN THE USA BY	Distributed in the USA by
INFORMATION FOR USA ONLY	Information needed for United States of America only
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
MICROPARTICLES	Microparticles
MULTI-ASSAY MANUAL DILUENT	Multi-Assay Manual Diluent
PRE-TRIGGER SOLUTION	Pre-Trigger Solution
PRODUCT OF IRELAND	Product of Ireland
REACTION VESSELS	Reaction Vessels
REAGENT LOT	Reagent Lot
REF	List Number
REPLACEMENT CAPS	Replacement Caps
SAMPLE CUPS	Sample Cups
SEPTUM	Septum
SN	Serial number
TRIGGER SOLUTION	Trigger Solution
WARNING: SENSITIZER	Warning: May cause an allergic reaction.
WASH BUFFER	Wash Buffer

The following US Patents are relevant to the ARCHITECT iSystem or its components. There are other such patents and patent applications in the United States and worldwide.

5,468,646	5,543,524	5,545,739
5,565,570	5,669,819	5,783,699

Abbott Ireland
Diagnostics Division
Lisnamuck, Longford
Co. Longford
Ireland
+353-43-3331000

CE

DISTRIBUTED IN THE USA BY

Abbott Laboratories
Abbott Park, IL 60064 USA

Customer Service: Contact your local representative or find country-specific contact information on www.abbottdiagnostics.com

Revised October 2015.

Abbott